JHR 94: 1–11 (2022)
doi: 10.3897/jhr.94.93555
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Host plant and immature stages of *Setabara histrionica* (MacGillivray) (Hymenoptera, Tenthredinidae)

Quinlyn Baine[1], David R. Smith[2], Bill Zakopyko[3], Sapphitah Dickerson[4], Chris Looney[4]

1 *Department of Biology, University of New Mexico, MSC03-2020, Albuquerque, NM 87131, USA* **2** *Department of Entomology, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, MRC-168, Washington, DC 20013, USA* **3** *Renton, WA, USA* **4** *Washington State Department of Agriculture, 1111 Washington St SE, Olympia WA, 98504, USA*

Corresponding author: Chris Looney (clooney@agr.wa.gov)

Academic editor: Marko Prous | Received 16 August 2022 | Accepted 21 November 2022 | Published 20 December 2022

https://zoobank.org/188516A1-C4DD-41FF-B08B-86B0BF9A77CF

Citation: Baine Q, Smith DR, Zakopyko B, Dickerson S, Looney C (2022) Host plant and immature stages of *Setabara histrionica* (MacGillivray) (Hymenoptera, Tenthredinidae). Journal of Hymenoptera Research 94: 1–11. https://doi.org/10.3897/jhr.94.93555

Abstract

The North American sawfly *Setabara histrionica* (Tenthredinidae: Heterarthrinae) is previously known only from adult collections but has been hypothesized to feed upon trees in the genus *Prunus*. We discovered a population of leaf-mining sawflies in Washington on *Prunus emarginata* and identified it as *S. histrionica* using combined morphological and molecular analysis. We observed preference in oviposition site selection on the host plant, with most eggs deposited on the margin of the basal third of the leaf, and on leaves growing within 1 meter of the ground. We include a description of the egg, larval stages, mine and phenology of *S. histrionica*, and an update to Smith's (1971) key to Heterarthrinae larvae.

Keywords

Heterathrinae, Leaf miner, *Prunus*, sawfly, Symphyta

Introduction

Setabara Ross, 1951 is a genus of small leaf-mining sawflies in the subfamily Heterarthrinae (Hymenoptera: Symphyta: Tenthredinidae) comprising three described species. *Setabara clypeiambus* Saini & Ahmad, 2013, is known from Arunachal Pradesh, India (Saini and Ahmad 2013), *S. sinica* Wei & Niu, 2014, from Zhejiang, China (Wei and Niu 2014), and *S. histrionica* (MacGillivray, 1909), from western North America (Smith 1971). *Setabara histrionica* is currently known from CA, CO, ID, NV, OR, and WA in the United States (Smith 1971) and BC and MB in Canada (Goulet and Bennett 2021). The host plants have not been confirmed for any of these species, although *Prunus* has long been suspected of being the host of *S. histrionica* based on adult collection data (Smith 1971).

In May 2014, we observed numerous adults of a small black sawfly swarming and mating in a thicket of *Rubus armeniacus* Focke (Himalayan blackberry) and *Prunus emarginata* (Douglas ex Hook.) D. Dietr. (bitter cherry) in a park in Redmond, WA (the *Prunus* species was confirmed using keys in Hitchcock and Cronquist 2018). While the sawflies were initially seen alighting upon blackberry leaves, upon closer examination we determined that this seemed to be out of convenience and numerous females were actually observed ovipositing on the *P. emarginata* leaves. Several specimens were collected and subsequently identified as *S. histrionica* (using Smith 1971), providing strong evidence that *Prunus* spp. is indeed a host genus for this species. We visited the site several times in following years to observe oviposition behavior, phenology, and larvae. Our observations of elements of the species' life history are described herein.

Methods

Phenology

Observations and collections were made in Marymoor Park, Redmond, WA (King County), at the head of the Sammamish River at the north end of Lake Sammamish. The site is located on the northern end of an off-leash dog area of the park and comprises a fairly large stand of *P. emarginata* trees bordered by a gravel parking lot and an open grassy field (47.6599, -122.1114). We visited the site periodically between 2015 and 2020. At each visit the area was surveyed for sawflies, and a selection was collected. We also looked for evidence of leaf mines at these visits. Adult specimens were identified in the lab using Smith (1971).

Oviposition activity

Oviposition of *S. histrionica* was assessed by collecting leaves at varying heights of *P. emarginata*. In 2015, leaves were collected by haphazardly selecting four trees in the stand and clipping branches from three heights: low (<0.75 m), medium (approximately 2 m), and high (2.5–3 m). In 2020 we repeated this sampling with two modifications. Trees were sampled on three transects through the stand: one along the north side, one through the center and one along the south side. Collections were made from

every tree within 1 meter of each transect, by haphazardly selecting a single branch from each of four heights - < 1 m, 1.5 m, 3 m, and ≥ 5 m from the ground. In both years every collected leaf was examined, the number of eggs or mines was counted, and their location on the leaf was recorded. Egg or mine location was approximated by dividing the leaf into thirds, (basal, middle, apical), and indicating if the egg was on the margin, along a vein, or along the midvein.

Mine and larval development

We photo-documented mines at the collection site between 30 May and 15 June, 2018. On 15 May, 2020, we collected several *S. histrionica* adults and transferred them to Olympia WA, where they were placed in six groups of three (1 female to 2 males) in mesh bags on short branches (~15 cm) of potted *P. emarginata* plants. Two of the six groups resulted in successful oviposition of 5 and 9 eggs, respectively. The number of eggs on each leaf was counted and the location on the leaf was recorded. After the eggs hatched, mines were examined twice a week to track development. Larvae were sacrificed periodically and preserved in 70% EtOH. Upon each sacrificial event the mine was dissected and searched for head capsules to determine the number of instars up to that point. Measurements of larvae (n = 30) and discarded head capsules (n = 29) found within captive-reared (n = 9) and field-collected mines (n = 50) were made under a Leica MXC microscope using the Leica image software package. Five larvae were left to develop in the bagged mines.

Molecular data

Two specimens from the Marymoor collections, one larva and one adult, were selected for mitochondrial *cytochrome oxidase subunit I* (COI) sequencing sequencing. We were also provided with a single dead larva from a leaf mine on *Prunus subcordata* Benth. (Klamath plum) collected near Bass Lake, California, in June of 2021 (V. Albu 2021, pers. comm.). DNA from the whole larval bodies, and three legs from the adult, was extracted in 5 µl to 30 µl extraction volumes with 10% Chelex, and 4% Proteinase K (20 mg/mL). Samples were incubated at 56 °C for 1 hour, then at 99 °C for 20 min. Each 25 µl PCR reaction contained 5 µl of molecular grade water, 12.5 µl of 2X Platinum II Hot-start Green PCR Master Mix (Invitrogen), 1.25 µl of $MgCl_2$ (50 mM), 0.5 µl of F and R primers (10 µM), and 2 µl of DNA template. For the larval extraction, the primer pair LCO1490 and HCO2198 (Folmer et al. 1994) with M13 tails amplified the COI barcode region with cycling conditions as follows: initial denaturation at 95 °C for 1 min., 35 cycles of denaturation at 96 °C for 2 seconds, annealing at 50 °C for 5 seconds, and extension at 72 °C for 20 seconds. Final extension was at 72 °C for 2 minutes. Because adult sample PCR amplification using above primers was unsuccessful, the adult extraction was amplified using primer pair LepF1 and LepR1 (Hebert et al. 2004) with cycling conditions as follows: initial denaturation at 94 °C for 3 min., 40 cycles of denaturation at 94 °C for 20 seconds, annealing at 50 °C for 20 seconds, and extension at 72 °C for 30 seconds. Final extension was at 72 °C for 5 minutes. Amplification controls included a non-template control containing molecular grade water, and a positive control containing *Lymantria dispar dispar*

DNA. Products were visualized via gel electrophoresis using a 1.5% agarose + 1X TBE gel at 160 Volts for 35 minutes. Amplicons of expected size were purified enzymatically using ExoSAP-IT Express (Applied Biosystems) kit per the manufacturer's protocol. Samples were sequenced in both forward and reverse directions using the Big Dye Xterminator cycle sequencing kit v3.1 and BigDye Xterminator BDX clean up kit (Applied Biosystems) on the ABI SeqStudio following manufacturer guidelines. All sequences were manually trimmed for quality to 658 bp using Geneious Prime 2021.2, then aligned using MUSCLE 3.8.425 with 10 iterations. All resulting sequences were submitted to NCBI repository (Washington: ON181656 and ON181654, California: ON181655).

Results

Phenology

Setabara histrionica appears to be univoltine. Adults were active for a period of about 2 weeks during the spring, based on weekly visits to the site between mid-April and late May in 2018, 2019, and 2020 (Table 1). The earliest observations of adults were made on 5 May, 2014, and the latest were 23 May, 2018. Most adults were observed and captured in the understory, although a few adults were also collected by sweeping taller *P. emarginata* within reach (~3m). Over 2/3 of the specimens captured were males, and males outnumbered females at every collecting event except one.

The first mines became visible about two weeks after adult activity was observed. Because of the semi-cryptic nature of leaf-mining larvae, the precise number of days spent in each developmental stage was not recorded. The average number of days from egg hatch to final instar in our captive sawflies was 41 (n = 5). All of these specimens were lost during the final instar. It seems likely that the larvae escaped the mesh rearing bags, perhaps by chewing their way through or by squirming through small creases where the bag was secured to the *Prunus* limbs. Soil from the captive pots was sieved and examined, but no pupae or pre-pupae were located.

Oviposition preference

We collected 61 branches and 489 leaves in 2015, and recorded the location of 292 eggs. In 2020 we collected 114 branches and 1201 leaves, but located only 36 eggs.

Table 1. Collection dates for adult *Setabara histrionica* at Marymoor Park, Washington.

Collection date	male	female
5 May 2014	9	5
14 May 2018	4	2
23 May 2018	2	0
16 May 2019	1	1
15 May 2020	15	7
18 May 2020	4	0

The majority of eggs discovered in both years were located in the lower tree branches (Table 2), with 61% were found within 1 m of the ground. All observed eggs were deposited on the margin of the leaf. Egg frequency decreased from the leaf stem towards the apex of each leaf, with more eggs located in the basal third than the central third, and more in the central third than in the apical third (Table 2).

Larval description

The habitus is typical of leaf-mining Heterarthrinae (Smith 1971): head and body slightly dorsoventrally flattened, prolegs reduced, prognathous. Body white to cream-white. Head capsule slightly wider than long. Larval measurements are reported as averages.

Egg: less than 1 mm in diameter, round or slightly oblong, appearing as a light green blister on the outside of the leaf (Fig. 1).

First instar: body length (n = 3): 2.38 mm, head capsule width (n = 13): 0.38 mm. Head capsule beige to amber, thoracic legs white. Prolegs present on abdominal segments 2–8.

Second instar: body length (n = 2): 2.59 mm, head capsule width (n = 12): 0.51 mm.

Third instar: body length (n = 12): 5.06 mm, head capsule width (n = 18): 0.63 mm.

Final instar: body length (n = 17): 6.70 mm, head capsule width (n = 16): 0.75 mm (Figs 2, 3). Head dark brown, body mostly white with various dark plates. Head: Head capsule dark brown, lighter ventrally and around mouthparts; antenna 2-segmented; each mandible with 3 sharp apical teeth; maxillary palpus with 4 palpomeres, lacinia with row of about 10 subequal short spines; epipharynx with transverse row of about 10 spines on each half; labial palpus with three palpomeres. Thorax: Thoracic legs, large rectangular plate on pronotum, narrow plate on anterior margin of mesonotum, large rectangular plate on prosternum, small central plates on mesosternum and metasternum dark brown; thoracic legs 5 segmented; coxae without tubercles; tarsal claws present, simple. Abdomen: Small central dark brown plate on sternum of first segment (smaller than metasternal plate); prolegs present on segments 2–8 and 10, each proleg with anterior crescent-shaped dark spot, anal proleg with complete, circular dark spot; sometimes tiny supraspiracular spot present.

Table 2. Oviposition sites of *Setabara histrionica* on *Prunus emarginata* leaves.

Branch height from ground	Leaves sampled	Eggs in basal 1/3 leaf	Eggs in middle 1/3 of leaf	Eggs in apical 1/3 of leaf	Mean eggs/leaf
2015					
<0.75 m	173	93	66	19	1.03
~ 2m	158	29	17	5	0.32
2.5–3 m	158	39	18	6	0.4
2020					
<1 m	351	9	10	3	0.06
~1.5 m	397	4	1	0	0.01
~3 m	270	4	1	0	0.02
> 5m	183	1	3	0	0.02

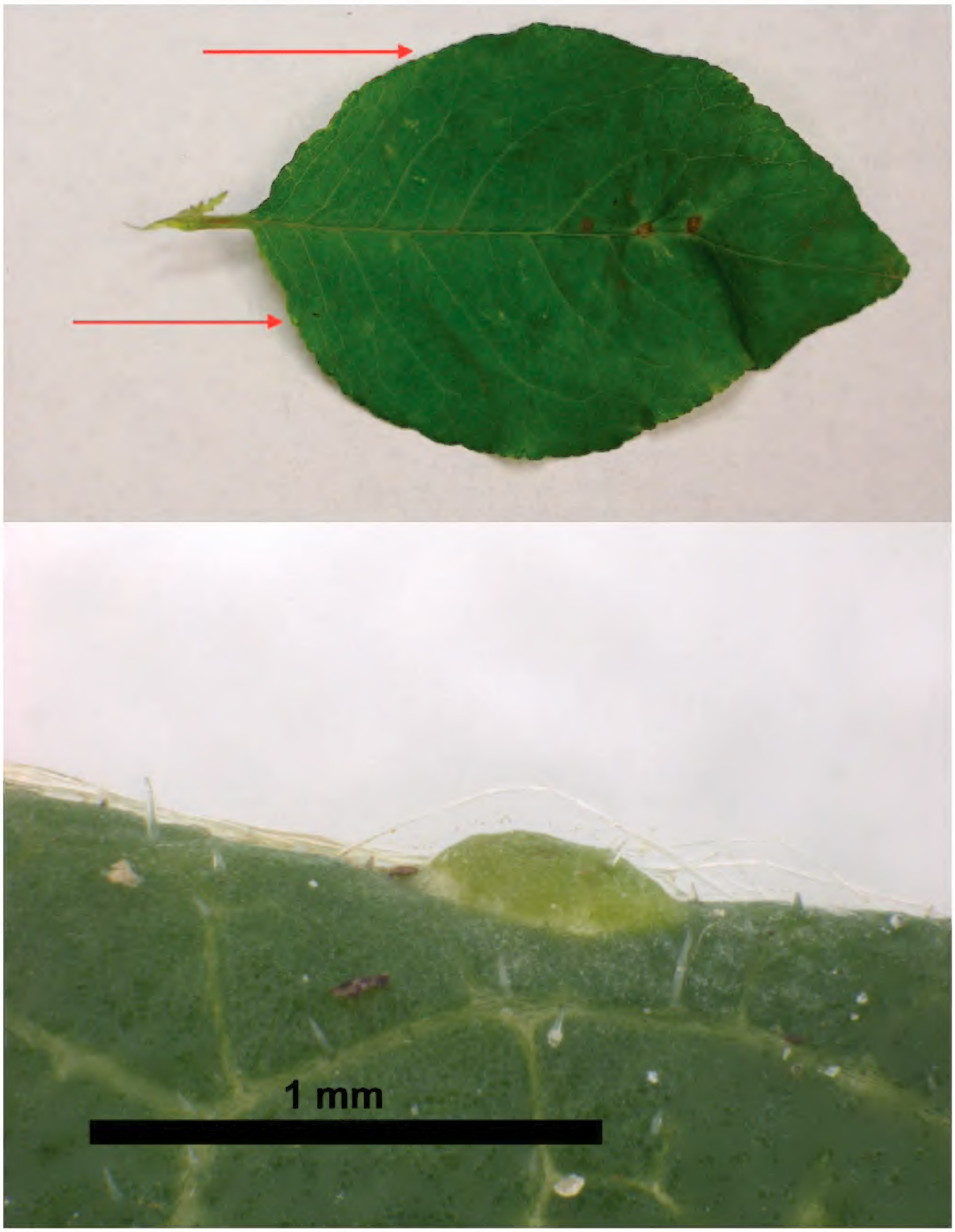

Figure 1. Location of *Setabara histrionica* eggs on a *Prunus emarginata* leaf, and close up of egg.

In Smith's 1971 key to Heterarthrinae larvae, *Setabara* goes to couplet 6 which includes *Messa* (now *Fenusella*) and *Profenusa* (in part). The key can be amended as follows:

6 Mesosternum, metasternum, and sternum of first abdominal segment with dark plates (pI. XII, 152, 153; pl.XIV, 164) **6A**

– Mesosternum, metasternum, and sternum of first abdominal segment without plates; on *Crataegus* (pI. XVI, 201, 202) .... ***Profenusa*** **MacGillivray (pt.)**

6A On *Populus*, *Betula*, *Salix*; 9th sternum with pair of small sound dark spots; Transcontinental; Smith 1971: fig. 153..... ***Fenusella*** **Leach (in key as *Messa*)**

– On *Prunus*; 9th sternum without dark spots; western (Fig. 3)..... ***Setabara histrionica***

Mine: After hatching, the larva feeds on leaf tissue in a semi-circular shape moving away from the leaf margin (Fig. 4). Mines continue to be excavated in a blotch pattern, often constrained by the midvein of the leaf, unless near the thinner area at the apex. Frass was distinctly oblong and capsule-shaped, and was broadly dispersed throughout the mine (Fig. 5). The average area at larval maturity was 15.49 mm^2 (n = 11). Pre-pupae exited the mine through a chewed arced slit in the cuticle, which measured 2.13 mm on average (n = 4) (Fig. 6).

Molecular analysis

The Washington specimens collected in this study were compared with two 421 bp COI sequences from *S. histrionica* specimens housed at the US National Museum of Natural History (USNM), also collected from Marymoor Park (BioProject PRJNA540960, GenBank accessions MW983660 and MW982369). All four trimmed Washington sequences were 100% identical to one another. In contrast, the sequence from the California larva was 95.3% identical to these, with 29 non-identical sites.

Figure 2. Fourth instar, head.

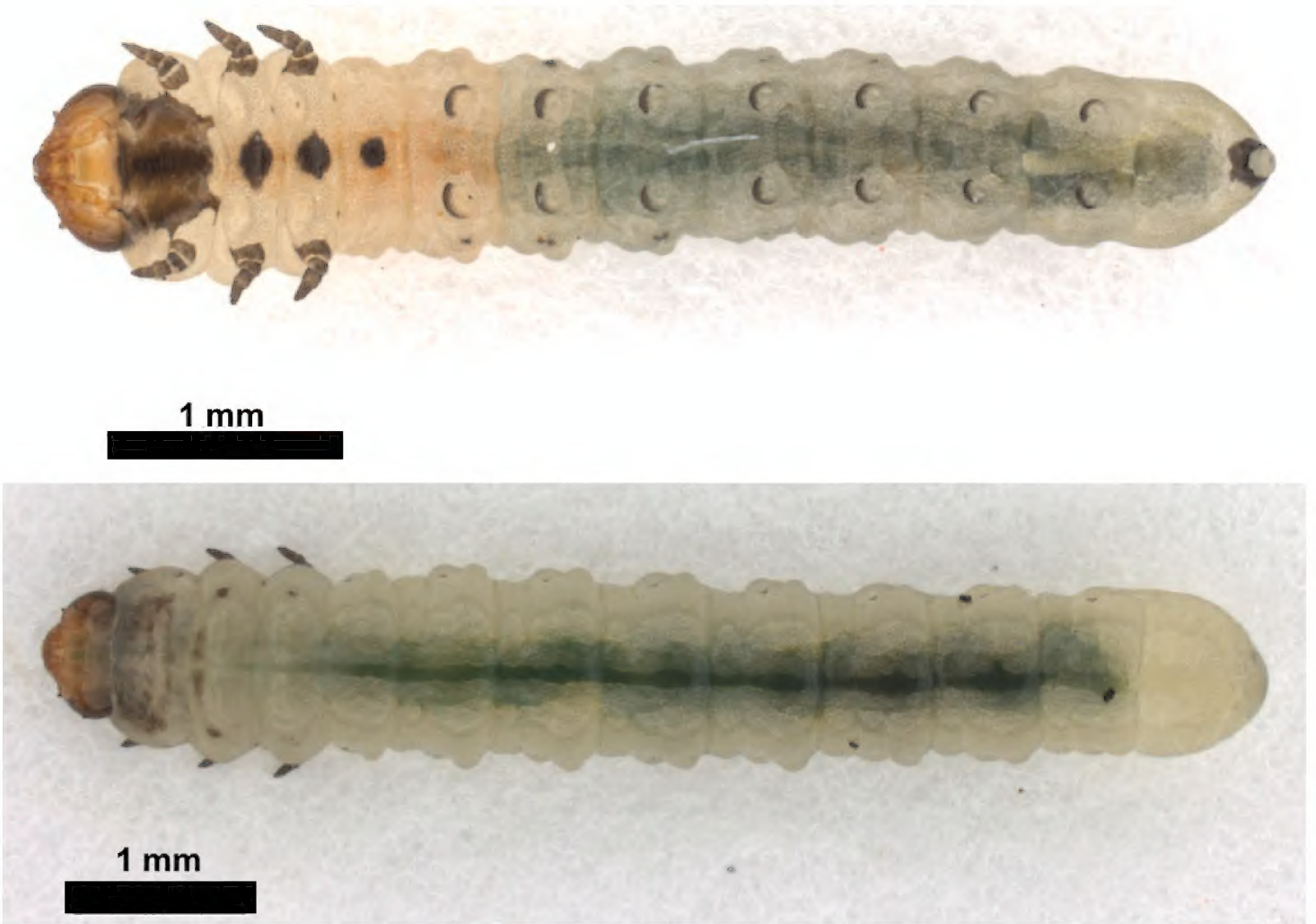

Figure 3. Fourth instar, ventral/dorsal view.

Figure 4. Mines at different stages of development.

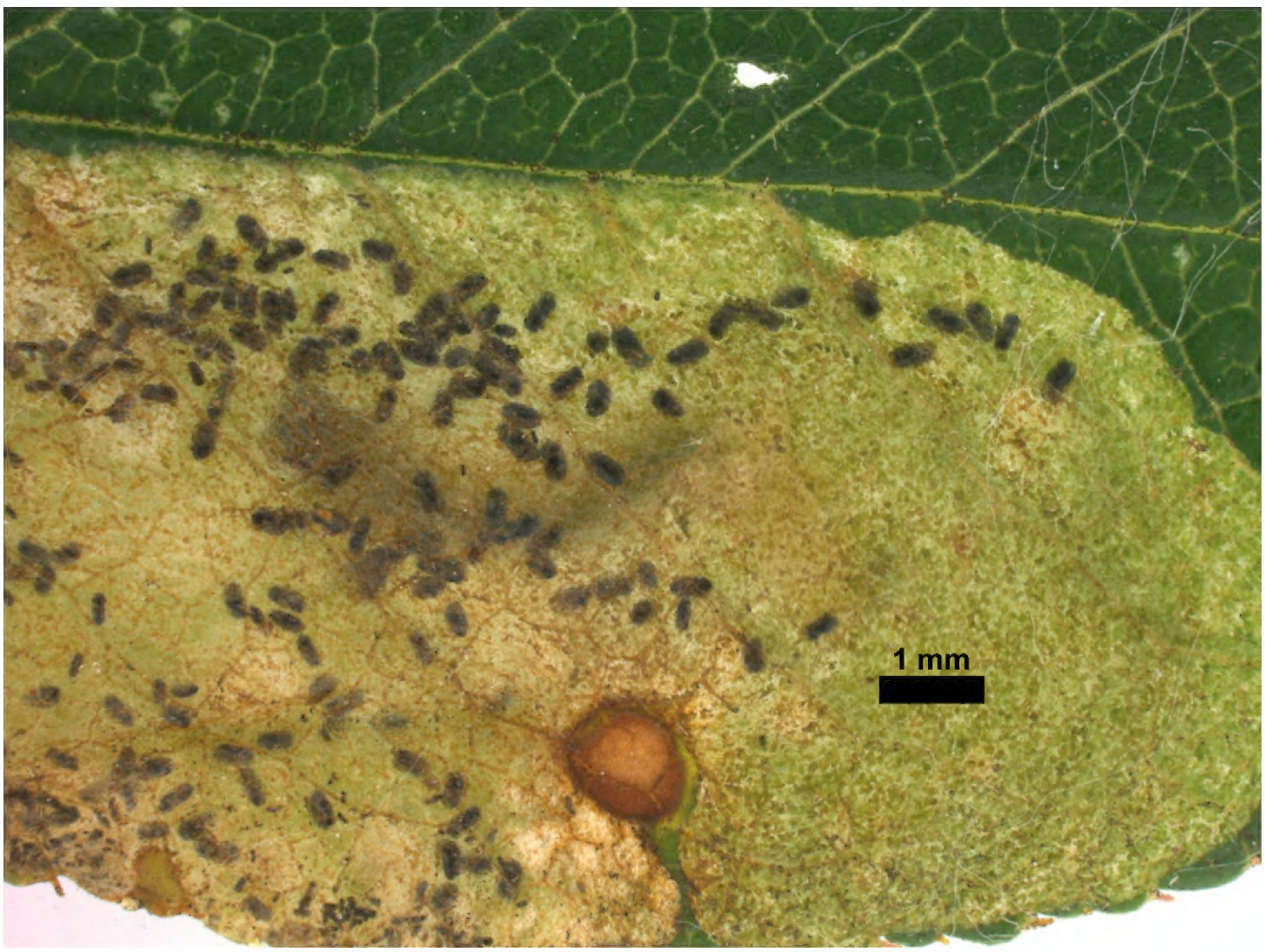

Figure 5. Frass shape and distribution, taken from late instar mine.

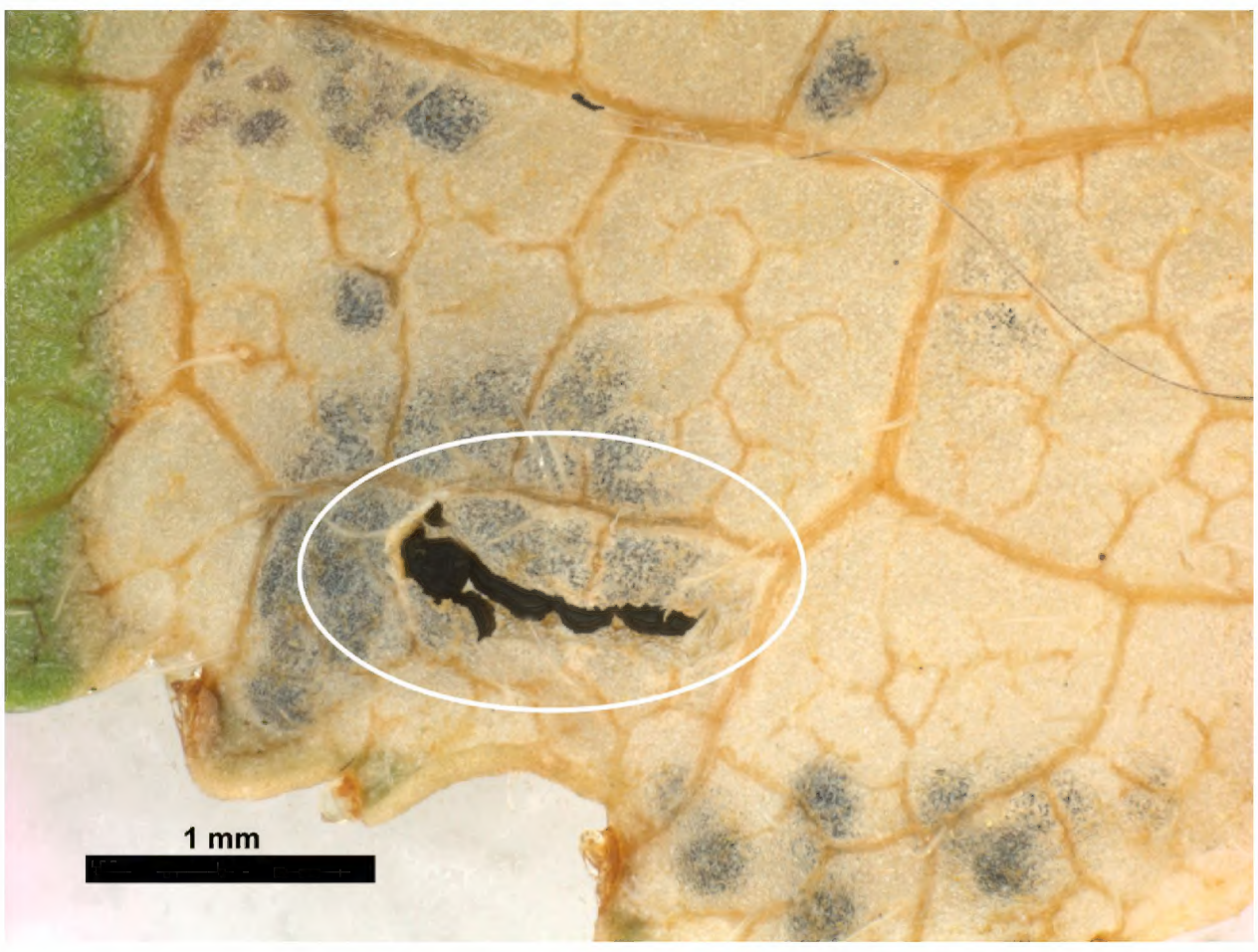

Figure 6. Pre-pupal emergence hole.

Discussion

Larvae of *S. histrionica* are similar in morphology and mining habits to those of the closely-related genus *Fenusella*, species of which mine *Populus*, *Salix*, and *Betula* (Underwood and Titus 1968; Smith 1971). *Metallus* are also very similar and are associated with species of Rosaceae, although not *Prunus* (Eiseman and Smith 2017). Both *Fenusella* and *Metallus* create blotch mines starting from the leaf margin, and exit the mine at maturity to overwinter in the soil below (Underwood and Titus 1968; Smith 1971; Eiseman and Smith 2017), as we observed in *S. histrionica*.

Adults have been collected from *Prunus subcordata*, in Oregon in May, 1964, (Smith 1971), in California from a mixed stand of *P. subcordata* and *P. virginiana* (V. Albu 2021, pers. comm.), and on *P. emarginata* (this study). Eiseman (2019) documents mines on *P. virginiana* in Colorado that appear similar to the mines we observed on *P. emarginata*. However, while the mine shape is similar, the frass within that mine is distinctly long and thin, rather than the capsule-shaped frass we observed in Washington. This difference could be a function of host plant, or the product of a different leaf-mining insect. This subtle difference in mine characteristics and the relatively large difference in COI sequence between the California and Washington specimens raise the possibility that there are two North American species of *Setabara*. This will be explored in future work - the adult specimens collected in California also key to *Setabara*, but a detailed comparison of the different populations has not been made.

One surprising aspect of these data was the heavily male-biased adult collections. Males of many Heterarthrinae are rarely collected, and several species are parthenogenetic. In contrast, reported sex ratios for *Fenusa pumila* and *Heterarthrus vagans* are at, or close to, parity (Digweed et al. 2009; Humble 2010). It is entirely possible that the sex ratio we observed is an artifact of haphazard sampling, rather than an actual aspect of the species' biology. Even so, this is another intriguing possibility that could be explored in greater detail.

Prior to this study, *S. histrionica* was only known from adult specimens and suspected to use *Prunus* as a host plant. These data confirm one host species, *Prunus emarginata*, and are the first description of the egg, larva, mine, and phenology for the genus. Many aspects of life history and morphology are similar to other leaf-mining Heterarthrinae. The potential existence of an undescribed species, and possibility of an unusual sex ratio, indicates that even in this small group discoveries remain to be made in North America.

Acknowledgements

The authors would like to thank the staff of Marymoor Park, Redmond WA, for access to the field site. Telissa Wilson (WSDA) assisted with molecular analysis, and Maggie Freeman (WSDA/ Oregon State University) helped with field work. We are grateful to Valeriu Albu for contacting us and sharing specimens from California. We would also like to Spencer Monckton and an anonymous reviewer who provided valuable feedback and suggestions to improve this article

References

Digweed SC, MacQuarrie CJK, Langor DW, Williams DJM, Spence JR, Nystrom KL, Morneau L (2009) Current status of invasive alien birch-leafmining sawflies (Hymenoptera: Tenthredinidae) in Canada, with keys to species. The Canadian Entomologist 141(3): 201–235. https://doi.org/10.4039/n09-003

Eiseman CS (2019) Leafminers of North America. Self-published e-book, [clii +] 1857 pp.

Eiseman CS, Smith DR (2017) Nearctic species of *Metallus* Forbes (Hymenoptera: Tenthredinidae): biology and distribution. Proceedings of the Entomological Society of Washington 119(4): 551–564. https://doi.org/10.4289/0013-8797.119.4.551

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Goulet H, Bennett AMR (2021) Checklist of the sawflies (Hymenoptera) of Canada, Alaska and Greenland. Journal of Hymenoptera Research 82: 21–67. https://doi.org/10.3897/jhr.82.60057

Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences 101(41): 14812–14817. https://doi.org/10.1073/pnas.0406166101

Hitchcock CL, Cronquist A (2018) Flora of the Pacific Northwest: an illustrated manual. University of Washington Press, 750 pp.

Humble LM (2010) First North American records for *Heterarthrus vagans* (Hymenoptera: Tenthredinidae), a Palaearctic leafmining sawfly of alder. The Canadian Entomologist 142: 181–187. https://doi.org/10.4039/n09-071

MacGillivray AD (1909) A synopsis of the American species of Scolioneurinae. Annals of the Entomological Society of America 2: 259–271. https://doi.org/10.1093/aesa/2.4.259

Ross HH (1951) Tenthredinidae In: Muesebeck CFW, Krombein KV, Townes HK (Eds) Hymenoptera of America North of Mexico, Synoptic Catalog. U. S. Department of Agriculture, Agriculture Monograph No. 2: 22–64, 66–82.

Saini MS, Ahmad M (2013) First report of *Profenusa* Macgillivray & *Setabara* Ross with one new species of each genus from India (Hymenoptera: Symphyta: Tenthredinidae). Annals of Entomology 31(1): 15–20.

Smith DR (1971) Nearctic Sawflies. III. Heterarthrinae: Adults and larvae (Hymenoptera: Tenthredinidae). Technical Bulletin, U.S. Department of Agriculture 1420: 1–84.

Underwood GR, Titus FA (1968) Description and seasonal history of a leaf miner on poplar *Messa populifoliella* (Hymenoptera: Tenthredinidae). Canadian Entomologist 100: 407–411. https://doi.org/10.4039/Ent100407-4

Wei M, Niu G (2014) *Setabara* Ross, a genus new to China with description of a new species (Hymenoptera: Tenthredinidae). Entomological News 124(2): 98–102. https://doi.org/10.3157/021.124.0204